REMARKS

Claims 1 – 8, 14, 26, 30, 32, 38, 39, and 41 are currently pending in the application. Claims 26, 30, 32, 38, 39 and 41 were previously withdrawn from consideration. Claims 9 – 13, 15 – 25, 27 – 29, 31, 33 – 37, 40 and 42 – 44 were previously cancelled. Claims 1, 7, and 14 have been amended. Claims 45 - 47 have been added. No new matter has been added, support being found throughout the specification and from the pending claims.

Objections

The Examiner has objected to the oath or declaration. The Examiner has requested that a new oath or declaration in compliance with 37 CFR 1.67(a) identifying the application by application number and filing date is required. The Examiner points out that the filing date of the PCT US03/07380 should be 3-10-03 rather than 3-10-02. Applicants have made the appropriate correction and a new oath or declaration is being filed under separate cover.

The Examiner has objected to the disclosure for minor informalities. The Examiner argues that "the term 'CLAIMS' in line 1 of page 70 should be changed. Applicants have amended the specification and respectfully request withdrawal of the objection.

The Examiner has objected to the disclosure because "the number of sequence of SEQ ID Nos. 29 – 38 do not match the number of sequences in Figure 2A (and) there are only 2 nucleotide sequences in Figure 3A rather than 3 sequence of SEQ ID Nos. 39 – 41 (Office Action, p.4)."

Applicants direct the Examiner to the Amendment submitted on 3/24/2005 in response to the Notice to Comply With Requirements for Patent Applications Containing Nucleotide And/Or Amino Acid Sequence Disclosures mailed by the Office on January 24, 2005. In the Amendment submitted on 3/24/2005, Applicants amended the specification to read:

> Figures 2A-D show cloning steps in generating libraries of fusion molecules according to one aspect of the invention. Figure 2A (**SEQ ID NOS 29 - 38 respectively in order of appearance**) shows preparation of a nucleic acid encoding an insertion sequence (e.g., β-lactamase) for subsequent cloning steps. Figure 2B shows random insertion of the insertion sequence into acceptor sequences digested with a nuclease. Figure 2C shows a variation of the insertion method shown in 2B which comprises incremental truncation. Figure 2D is a flow chart illustrating selection of active fusions according to one aspect of the invention. (page 9, lines 23-29; emphasis added)

> Figures 3A-G illustrate methods of using molecular switches according to aspects of the invention. Figure 3A shows regulation of gene transcription using a fusion molecule according to one aspect of the invention (**SEQ ID NOS 39 - 41 respectively in order of appearance**). Figure 3B shows modulation of a cell signaling pathway according to another aspect of the invention. Figure 3C shows drug delivery mediated by a fusion molecule to a cell expressing a marker of a pathology. Figure 3D shows the use of fusion molecules for drug transport to an intracellular compartment. Figure 3E shows delivery of a conditionally toxic fusion molecule to a cell. Figure 3F shows the use of a fusion molecule for metabolic engineering. Figure 3G shows a fusion molecule according to one aspect of the invention which functions as a biosensor. (page 10, lines 1 – 10; emphasis added)

Applicants wish to point out to the Examiner that in Figure 2A, SEQ ID NO: 33 and SEQ ID NO: 36 appear more than once in Figure 2A.

Claim Rejections

35 U.S.C. §102(b)

The Examiner has rejected claims 1 – 8 and 14 under 35 U.S.C. §102(b) as being allegedly anticipated by Siegel et al (Methods in Enzymology, Vol.327, p.249 – 259, as cited on IDS). The Examiner argues that the Siegel et al. reference teaches "generation of a fusion protein by fusing GFP in-frame into the middle of a signal transduction protein (detector) so that conformational rearrangement in the detector perturbs the floursecence of GFP via the use of

expression vector having sequence encoding the GFP and signal transduction protein under the control of cell-specific promoter (Figure 1) (Office Action, p.5)." Applicants respectfully traverse the rejection.

The instant claims recite a method for assembling a modulatable fusion molecule. The method as instantly claimed comprises inserting randomly an insertion sequence into an acceptor sequence, wherein the insertion sequence and the acceptor sequence each comprise a state, and thereby generating a fusion molecule.

The Siegel reference, as pointed out by the Examiner, teaches generation of a fusion protein by fusion of GFP in-frame into the middle of a signal transduction protein. The Siegel reference does not teach or suggest a method for assembling a modulatable fusion molecule as taught by the instant claims. There is no teaching or suggestion in the Siegel reference that the insertion is **random**, and there is no teaching or suggestion in the Siegel reference that any random insertion occurs. In fact, the Seigel reference merely teaches a Shaker K+ sequence with a point mutation fused with a GFP protein. There is no teaching or suggestion in the Siegel reference that the site of point mutation is the site of insertion of the (GFP) insertion sequence. Thus, according to the teachings of Siegel, to produce a functional GFP based sensor, insertion of the GFP sequence **cannot occur in a random manner** as set forth by the instant claims, but rather occurs in certain **specified positions** to assemble a fusion protein.

Thus, the Seigel reference does not provide any teaching or suggestion towards a method comprising **randomly inserting** an insertion sequence into an acceptor sequence. Applicants respectfully request that the rejection be reconsidered and withdrawn.

The Examiner has rejected claims 1 – 8 under 35 U.S.C. §102(b) as being allegedly anticipated by Kratz et al (PNAS, Vol.96, pp.1915 - 1920). The Examiner argues that the Kratz et al. reference teaches "preparation of a DNA

construct for producing GFP fusion protein, wherein the entire GFP sequence, flanked on both sides by Gly-rich linkers, is inserted into the central c/el epitope of the truncated, assembly-competent core protein derivative core 1 – 149 of hepatitis B virus (HBV)" and that "(g)eneration of the GFP fusion protein constitutes insertion of an insertion sequence into an acceptor sequence and said insertion couples the state of the insertion sequence to the state of the acceptor sequence (Office Action, p.7)." Applicants respectfully traverse the rejection.

As pointed out by the Examiner, the Kratz reference merely teaches "preparation of a DNA construct for producing GFP fusion protein, wherein the entire GFP sequence, flanked on both sides by GLY-rich linkers, is inserted into the central c/el epitope of the truncated, assembly-competent core protein derivative core 1 – 149 of HBV (Office Action, p.7)." The Kratz reference does not teach a method comprising randomly inserting an insertion sequence into an acceptor sequence. The Examiner argues that "GFP can be considered as an insertion sequence and the HBV core protein can be considered as an acceptor sequence, which has a deletion (Office Action, p.7);" however nowhere in the Kratz reference is there teaching or suggestion that the GFP sequence is inserted into the acceptor HBV sequence at a **random point** or in a **random manner**. Nowhere in the Kratz reference is there teaching or suggestion of insertion of an insertion sequence into an acceptor sequence as taught by the instant claims. Rather, the Kratz reference teaches that the insertion sequence is **specifically and preferably** inserted at the c/e1 epitope of HBV (the acceptor).

Thus, the Kratz reference does not provide any teaching or suggestion towards a method for assembling a modulatable fusion molecule as taught by the instant claims. Applicants respectfully request that the rejection be reconsidered and withdrawn.

The Examiner has rejected claims 1 – 8 and 14 under 35 U.S.C. §102(b) as being allegedly anticipated by Lacatena et al (PNAS, Vol.91, pp. 10521 – 10525). The Examiner argues that the Lacatena et al. reference teaches "using TnphoA to generate human beta2-adrenergic receptor (hubeta2AR)-phoA fusion protein in vivo by transposition of TnphoA into the hubeta2AR gene in PUC18 (Office Action, p.8)" and that "phoA can be considered as an insertion sequence and the hubet2AR can be considered as an acceptor sequence, which has a deletion (Office Action, p.8)." Applicants respectfully traverse the rejection.

As pointed out by the Examiner, the Lacatena reference teaches "using TnphoA, a transposon probe for protein export signals, to generate hubeta2AR-phoA fusion protein in vivo by transposition of TnphoA into the hubeta2AR gene in PUC18 (Office Action, p.8)." The Lacatena reference specifically teaches a method of generating "hubetaAR-PhoA fusions by transposition of Tn*phoA* into the hubetaAR gene (p.10522)." The method taught by Lacatena et al. is specific, and does not teach or suggest that the transposition of TnphoA into the hubeta2AR gene occurs at a **random position** or in a **random manner**. As shown in figure 2 and the accompanying text (p.10522- 23), specificity in the location of the hubeta2AR-PhoA fusion affected alkaline phosphatase activity (a measure of PhoA activity, see e.g. Figure 2), and moreover the enzymatic activities of the fusions differed dependent upon the point of gene transposition. Accordingly, Applicants respectfully request that the rejection be reconsidered and withdrawn.

The Examiner has rejected claims 1 – 8 and 14 under 35 U.S.C. §102(e) as being allegedly anticipated by Anderson et al (US Patent No. 6,596,485 B2; "the '485 patent"). The Examiner argues that the '485 patent teaches generating various fusion proteins by fusing GFP or its derivatives or variants into random peptides in such a manner that the structure of the GFP is not significantly perturbed and the peptide is metabolically conformationally stabilized" and that "(g)eneration of the GFP fusion protein constitutes insertion of an insertion

sequence into an acceptor sequence and said insertion couples the state of the insertion sequence to the state of the acceptor sequence (Office Action, p.9)." Applicants respectfully traverse the rejection.

The Examiner argues that "the peptide can be considered as an insertion sequence and the GFP can be considered as an acceptor sequence, which can have a deletion, a substitution or insertion (Office Action, p.9)." The Examiner argues that "the inducible promoter, such as Tet regulatory element, is responsive to inducer, such as tetracycline (and) when inducer...is present, the fusion molecule switches state in response to the signal (Office Action, p.9)."

The '485 patent, however, merely teaches a GFP fusion protein, where "a random peptide (is) fused to green fluorescent protein (col 2, ln 8 – 9)." The '485 application **does not teach** that insertion of the insertion sequence (the peptide) into the acceptor sequence (GFP) is **random**, but rather that "(p)referred embodiments utilize fusions to the N- and C-termini of GFP comprising presentation structures capable of presenting the peptide in a conformationally restricted form. Further preferred embodiments fuse the random peptide to an internal position of GFP, including the loops comprising amino acids 130 to 135, amino acids 154 to 159, amino acids 172 to 175, amino acids 188 to 193, amino acids 208 to 216 (col 2, lines 9 – 16)." The '485 patent teaches particular positions in the acceptor sequence where insertion of the insertion sequence is preferred; this is not **inserting randomly**.

Thus, the '485 patent does not provide any teaching or suggestion towards a method for assembling a modulatable fusion molecule as taught by the instant claims, wherein insertion couples the state of the insertion sequence to the state of the acceptor sequence after a **random** double stranded break in DNA. Applicants respectfully request that the rejection be reconsidered and withdrawn.

The Examiner has rejected claims 1 – 8 and 14 under 35 U.S.C. §102(b) as being allegedly anticipated by Doi et al (FEBS Letters, Vol.453, p.305 - 307).

The Examiner argues that the Doi reference "teaches generation of GFP-based sensors by inserting a desired molecular-recognition domain into a loop of GFP (and) in the absence of the target molecule, conformational fluctuations of the inserted domain put stress on the GFP scaffold with a consequent reduction in fluorescence (Office Action, p.10)."

The subject matter of the instant claims is discussed above. The Examiner argues that "Doi teaches preparation of expression vector expressing GFP-TEM1 beta-lactamase (GFP-Bla) fusion protein with Bla inserted between Gln-172 and Asp-173 of GFP. The Examiner points out that Doi teaches "random mutagenesis of the fusion GFP:Bla gene (that) was performed using error-prone PCR (Office Action, p.10)." The beta-lactamase fusion taught by Doi et al. comprises an insertion of the beta-lactamase inhibiting protein (BLIP) into a surface loop of the GLP protein. Contrary to the teachings of the instant invention, Doi et al. teaches that **several rounds** of random mutagenesis must be performed in order to obtain polypeptides with increased fluorescence upon binding of ligand to BLIP.

Thus, the Doi et al. reference does not provide any teaching or suggestion towards a method for assembling a modulatable fusion comprising random insertion of an insertion sequence into an acceptor sequence. Applicants respectfully request that the rejection be reconsidered and withdrawn.

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Respectfully submitted,

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